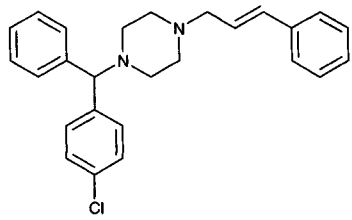
Clocinizine



Molecular formula: $C_{26}H_{27}ClN_2$
Molecular weight: 402.97
CAS Registry No.: 298-55-5
Merck Index: 2428

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 20.432

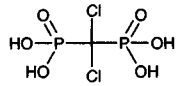
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clodronic acid



Molecular formula: $CH_4Cl_2O_6P_2$
Molecular weight: 244.89
CAS Registry No.: 10596-23-3
Merck Index: 2432

SAMPLE

Matrix: blood, solutions, tissue
Sample preparation: Serum. Add 250 μL human serum to 250 μL MeOH, mix, centrifuge. Evaporate 300 μL aliquot of the supernatant to dryness under a stream of nitrogen. Reconstitute the residue in 400 μL water. Inject a 20 μL aliquot. Tissue. Add 400 μL MeOH to 400 μL liver homogenate. Mix, centrifuge, evaporate a 450 μL aliquot of the supernatant to dryness under a stream of nitrogen at 20°. Reconstitute the residue with 600 μL water. Inject a 20 μL aliquot. Solutions. Prepare solutions in 50 mM pH 7.4 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Kromasil 100 RP-C8 (Higgins Analytical, USA)
Mobile phase: MeOH:buffer 3:97 (Buffer was 100 mM ammonium acetate containing 230 mM butylamine, adjusted to pH 4.6 with acetic acid.)
Flow rate: 1.2
Injection volume: 20
Detector: ELSD, Sedex 55 (Sedere, France), 70°, nebulizing gas (dried and filtered air) pressure 2.2 bar

CHROMATOGRAM

Retention time: 4.26
Limit of detection: 37.5 μg/mL
Limit of quantitation: 50 μg/mL

KEY WORDS

serum; liver; rabbit; human

REFERENCE

Niemi,R.; Taipale,H.; Ahlmark,M.; Vepsäläinen,J.; Järvinen,T. Simultaneous determination of clodronate and its partial ester derivatives by ion-pair reversed-phase high-performance liquid chromatography coupled with evaporative light-scattering detection, *J.Chromatogr.B*, **1997**, *701*, 97–102.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with water to a concentration of 850 μg/mL clodronic acid. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: AG5 (Dionex)
Column: 250 × 4 15 μm IonPac AS5 (Dionex)
Mobile phase: Gradient. 20 mM NaOH to 100 mM NaOH over 20 min
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: Conductivity, Dionex ED40, thermostated DS3 cell in conductivity mode, detection carried out using an Anion Self Regenerating Suppressor (ASRS-1) in recycle mode

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES
Simultaneous: carbonylbisphosphonic acid, chloride, diprotic acid, methylenebisphosphonic acid, monochloromethylenebisphosphonic acid, nitrate, phosphate, sulfate, triprotic acid

KEY WORDS
injections

REFERENCE
Taylor,G.E. Determination of impurities in clodronic acid by anion-exchange chromatography, *J.Chromatogr.A*, **1997**, *770*, 261–271.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water to a concentration of 400 μg/mL, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 6 μm IC-Pak HR anion-exchange (Waters)
Mobile phase: 12 mM nitric acid
Flow rate: 1
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 6
Limit of detection: 1000 ng/mL

KEY WORDS
injections; indirect UV detection; rugged

REFERENCE
Tsai,E.W.; Chamberlin,S.D.; Forsyth,R.J.; Bell,C.; Ip,D.P.; Brooks,M.A. Determination of bisphosphonate drugs in pharmaceutical dosage formulations by ion chromatography with indirect UV determination, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 983–991.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 30.4

OTHER SUBSTANCES
Simultaneous: alendronate, etidronate, neridronate, olpadronate, pamidronate

KEY WORDS
derivatization; complexation

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE
Matrix: urine
Sample preparation: Inject directly.

HPLC VARIABLES
Guard column: 50 × 4 10 μm HPLC AG7 anion-exchange (Dionex)
Column: 250 × 4 10 μm HPIC AS7 anion-exchange (Dionex)
Mobile phase: 30 mM nitric acid adjusted to pH 2.4 with dilute NaOH
Flow rate: 1
Injection volume: 100
Detector: UV 550 following post-column reaction. Column effluent with reagent pumped at 1 mL/min, allow to flow through 1.2 m × 0.25 mm i.d. PEEK tubing to the detector. (Reagent was 4.5 mL 0.2 mM thorium solution + 100 mL 0.4 mM disodium EDTA in water, mix, add 60 mL buffer, add 1.9 mL 500 μg/mL xylenol orange in water, shake vigorously, adjust pH so that the combined pH of the reagent and the column effluent will be 6.0-6.3, dilute to 500 mL with water, allow to stand for at least 1 h before use. thorium solution was 1.104 g $Th(NO_3)_3.4H_2O$ in 20 mL 2 M nitric acid, dilute to 500 mL with water to give a 4 mM solution, dilute an aliquot further to give a 0.2 mM solution. Buffer was 10.1 mL ethylenediamine in ice-cold water, add 15 mL concentrated HCl slowly with stirring so as to keep the temperature below 20°, adjust pH to 7.3 with dilute HCl, dilute to 250 mL with water.)

CHROMATOGRAM
Retention time: 12
Limit of detection: 1100 ng/mL

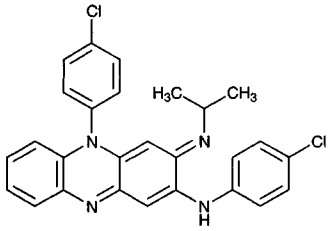
OTHER SUBSTANCES
Extracted: clodronate esters

KEY WORDS
post-column reaction

REFERENCE
Virtanen,V.; Lajunen,L.H. High-performance liquid chromatographic method for simultaneous determination of clodronate and some clodronate esters, *J.Chromatogr.*, **1993**, *617*, 291–298.

Clofazimine

Molecular formula: $C_{27}H_{22}Cl_2N_4$
Molecular weight: 473.40
CAS Registry No.: 2030-63-9
Merck Index: 2433



SAMPLE

Matrix: blood

Sample preparation: Condition a Prep Sep CN SPE cartridge (Fisher) with 3 mL MeOH, 5 mL water, and 5 mL 100 mM pH 6.0 phosphate buffer, do not allow to go dry. 1 mL Plasma + 100 µL MeOH:water 10:90 + 5 mL 100 mM pH 6.0 phosphate buffer, vortex, let stand, add to the SPE cartridge, air dry for 2 min, add 50 µL 3.4 µg/mL salicylic acid, elute with four 1 mL portions of THF:MeCN:MeOH 40:40:20 containing 0.7 mM hexanesulfonic acid. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 3.9 µBondapak C18
Mobile phase: MeOH:THF:0.5% acetic acid 5:40:55 containing 2.5 mM hexanesulfonic acid
Flow rate: 1.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 6
Internal standard: salicylic acid (3.3)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Simultaneous: dapsone

KEY WORDS

plasma; SPE; rabbit; pharmacokinetics; human

REFERENCE

Krishnan,T.R.; Abraham,I. A rapid and sensitive high performance liquid chromatographic analysis of clofazimine in plasma, *Int.J.Lepr.Other Mycobact.Dis.*, **1992**, *60*, 549–555.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Add 2 µg IS in dichloromethane to a glass tube. Evaporate by heating to 40°. Homogenize 100 mg tissue in 1 mL water. Pour the homogenate into the tube coated with IS, add 1 mL 5 M NaOH, add 2 mL dichloromethane, mix on a blood tube mixer for 20 min, sonicate for 5 min, centrifuge at 1800 g for 15 min. Discard the aqueous layer, add 1.5 mL 10% NaOH in EtOH to the organic layer, heat at 80° until the bubbling stopped (indicating complete evaporation of the dichloromethane), add 6 mL cold water, add 2 mL dichloromethane, mix for 5 min on a blood tube mixer, centrifuge at 1400 g for 10 min, discard the aqueous layer, wash the organic layer twice more in the same fashion. Remove a 1.1 mL aliquot of the organic layer and evaporate it to dryness by heating at 40° for 2 h. Reconstitute with 120 µL 0.6% (v/v) acetic acid in THF, inject a 20 µL aliquot. Serum. Add 2 µg IS in dichloromethane to a glass tube. Evaporate by heating to 40°. Add 1 mL serum, 1 mL 5 M NaOH, and 2 mL dichloromethane, mix on a

blood tube mixer for 20 min, sonicate for 5 min, centrifuge at 1800 g for 15 min. Remove a 1.1 mL aliquot of the organic layer and evaporate it to dryness by heating at 40° for 2 h. Reconstitute with 60 μL 0.6% (v/v) acetic acid in THF, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 300 × 3.9 10 μm Bondclone C18 (Phenomenex)
Mobile phase: THF:water:acetic acid 40:59.4:0.6 containing 471 mg/L hexanesulfonic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 5.6
Internal standard: B4090 (4.4)
Limit of quantitation: 10 ng/mL (serum, tissue) 20 ng/g (fat)

KEY WORDS

fat; serum; rat

REFERENCE

O'Connor,R.; O'Sullivan,J.F.; O'Kennedy,R. Determination of serum and tissue levels of phenazines including clofazimine, *J.Chromatogr.B*, **1996**, *681*, 307–315.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 NewGuard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4:triethylamine 61.5:0.5 adjusted to pH 4.2 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM

Retention time: 90

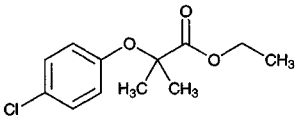
OTHER SUBSTANCES

Simultaneous: medazepam, rifabutin
Noninterfering: amikacin, isoniazid, streptomycin, zidovudine

REFERENCE

Lewis,R.C.; Hatfield,N.Z.; Narang,P.K. A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by high-performance liquid chromatography (HPLC), *Pharm.Res.*, **1991**, *8*, 1434–1440.

Clofibrate



Molecular formula: $C_{12}H_{15}ClO_3$
Molecular weight: 242.7
CAS Registry No.: 637-07-0
Merck Index: 2436
Lednicer No.: 1 119

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.267

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: tissue
Sample preparation: Powder rat liver under liquid nitrogen. Homogenize tissue with cooled 6% perchloric acid, centrifuge at 8000 g for 3 min, inject a 20-50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: MeOH:28 mM pH 4.2 phosphate buffer 52:48
Flow rate: 1.5
Injection volume: 20-50

Detector: UV 230

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 2 pmole

OTHER SUBSTANCES
Extracted: clofibroyl coenzyme A

KEY WORDS
rat; liver

REFERENCE
Lygre,T.; Aarsaether,N.; Stensland,E.; Aarsland,A.; Berge,R.K. Separation and measurement of clofibroyl coenzyme A and clofibric acid in rat liver after clofibrate adminstration by reversed-phase high-performance liquid chromatography with photodiode array detection, *J.Chromatogr.*, **1986**, *381*, 95–105.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 20-fold with 100 mM pH 2.0 phosphate buffer, extract twice with two volumes of ethyl acetate, centrifuge at 5000 g for 5 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen below 30°. Reconstitute in 0.2-1 mL mobile phase, inject an aliquot.

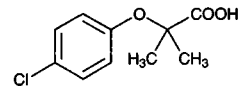
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 224

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

Clofibric acid



Molecular formula: $C_{10}H_{11}ClO_3$
Molecular weight: 214.65
CAS Registry No.: 882-09-7
Merck Index: 2437

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C2 SPE cartridge (Analytichem) with 2 mL MeOH and 1 mL water. 100 μL Plasma + 20 μL 200 μg/mL ketoprofen in MeOH + 500 μL 1 M HCl, vortex for 15 s, add to the SPE cartridge, rinse out tube with 1 mL water, add rinse to the SPE cartridge, elute with 1 mL mobile phase, vortex the eluate, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 30-40 μm pellicular Vydac Reversed-Phase
Column: 75 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeOH:buffer 42:58 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 7.0 with 50% aqueous NaOH.)
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4B, LC-17 thin-layer glassy carbon working electrode +1.10 V, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through an air-cooled 7.9 m × 0.3 mm ID PTFE coil irradiated by an SC3-9 ultraviolet lamp (UVP, Inc.) to the detector.

CHROMATOGRAM

Retention time: 2
Internal standard: ketoprofen (2.7)
Limit of detection: 200 ng/mL

KEY WORDS

post-column reaction; plasma; SPE; post-column photochemical derivatization

REFERENCE

Bachman,W.J.; Stewart,J.T. HPLC-photolysis-electrochemical detection in pharmaceutical analysis: Application to the determination of clofibric acid in human plasma, *J.Liq.Chromatogr.*, **1989**, *12*, 2947–2959.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 1 μg/mL propyl paraben in MeCN (if clofibric acid concentration is 15-60 μg/mL add 25 μL MeCN), shake gently, vortex for 30 s, centrifuge at 5000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: Adsorbosil C18 (Alltech)
Column: 150 × 4.6 5 μm Microsorb-MV
Mobile phase: MeCN:water:acetic acid 45:55:1
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 7.12

Internal standard: propyl paraben (5.56)
Limit of quantitation: 1.5 μg/mL

OTHER SUBSTANCES
Simultaneous: bezafibrate, clofibrate, fenofibrate, fenofibric acid, gemfibrozil

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Lau-Cam,C.; Theofanopoulos,V.; Spireas,S.S. Simplified HPLC method with spectrophotometric detection for the assay of clofibric acid in rat plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 3945–3954.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Plasma or 100 μL urine + 500 (plasma) or 300 (urine) mg NaCl + 1 (plasma) or 0.3 (urine) mL pH 4 buffer + 5 mL n-hexane:EtOH 90:10, shake horizontally for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, add 50 μL toluene and evaporate it to remove traces of water. Reconstitute the residue in 500 μL dichloromethane, add 50 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 50 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 50 μL 1 mg/mL FLOPA, vortex, let stand at room temperature for 2 h, evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 50 μL aliquot. (FLOPA is the corresponding amine hydrochloride from (+)-(S)-flunoxaprofen. Synthesis is as follows (protect from light). 500 mg (+)-(S)-Flunoxaprofen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, add 600 mg sodium azide dissolved in ice water with stirring, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give crystals (mp 91°). Dissolve in ether, add 0.5 M HCl in ether, filter, dissolve solid in a small volume of MeOH, precipitate with ether, dry FLOPA over phosphorus pentoxide under vacuum (Pharm.Res. 1990, 7, 1262).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Gradient. A was n-hexane:chloroform:EtOH 100:10:0.75. B was n-hexane:chloroform:EtOH 100:10:20. A:B 100:0 for 10 min, 50:50 for 5 min, 100:0 for 5 min (stepwise).
Flow rate: 2
Injection volume: 50
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 8
Internal standard: clofibric acid

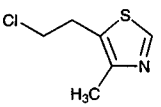
OTHER SUBSTANCES
Extracted: beclobrate

KEY WORDS
clofibric is IS; derivatization; normal phase; plasma

REFERENCE

Mayer,S.; Mutschler,E.; Spahn-Langguth,H. Pharmacokinetic studies with the lipid-regulating agent beclobrate: enantiospecific assay for beclobric acid using a new fluorescent chiral coupling component (S-FLOPA), *Chirality*, **1991**, *3*, 35–42.

Clomethiazole



Molecular formula: C_6H_8ClNS
Molecular weight: 161.65
CAS Registry No.: 533-45-9, 6001-74-7 (HCl), 1867-58-9 (ethanedisulfonate)
Merck Index: 2444

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 249.9

CHROMATOGRAM

Retention time: 15.958

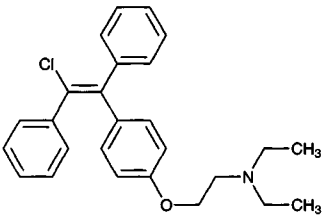
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clomiphene



Molecular formula: $C_{26}H_{28}ClNO$
Molecular weight: 405.97
CAS Registry No.: 911-45-5, 50-41-9 (citrate)
Merck Index: 2446
Lednicer No.: 1 105

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 1 mL pH 9 borate buffer, vortex, add 9 mL redistilled ether, vortex for 2 min, centrifuge at 1600 g for 15 min. Remove the organic layer and dry it over 1 g anhydrous sodium sulfate, centrifuge at 1600 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 75 μL mobile phase, vortex for 2 min, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 6 μm Zorbax Sil
Mobile phase: Chloroform:MeOH 80:20
Flow rate: 0.8
Injection volume: 10
Detector: F following post-column reaction. The column effluent flowed through a 3 m × 0.3 mm ID PTFE coil irradiated by a medium-pressure mercury lamp (Hanovia) with water cooling to the detector.

CHROMATOGRAM

Retention time: 7 (cis-clomiphene), 7.5 (trans-clomiphene)
Limit of detection: 350 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

post-column reaction; post-column photochemical derivatization; plasma; normal phase

REFERENCE

Harman,P.J.; Blackman,G.L.; Phillipou,G. High-performance liquid chromatographic determination of clomiphene using post-column on-line photolysis and fluorescence detection, *J.Chromatogr.*, **1981**, *225*, 131–138.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 2 mL diethyl ether, extract, centrifuge at 2000 rpm at 4° for 10 min, repeat extraction. Combine the organic phases and evaporate them to dryness under a stream of nitrogen at 37°. Reconstitute the residue in 250 μL MeOH, centrifuge at 2000 rpm at 4° for 10 min, inject a 10-100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:10 mM KH_2PO_4:300 mM phosphoric acid :water 42:20:10:28
Flow rate: 2.8
Injection volume: 10-100
Detector: F ex 258 em 378 following post-column reaction. The column effluent flowed through a 6.5 m × 0.35 mm × 1.5 mm o.d. PTFE tube irradiated with a Philips HPK 125 watt high pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 12
Internal standard: clomiphene (9)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: tamoxifen
Simultaneous: metabolites

KEY WORDS
plasma; clomiphene is IS; post-column reaction; post-column photochemical derivatization

REFERENCE
Milano,G.; Etienne,M.C.; Frenay,M.; Khater,R.; Formento,J.L.; Renee,N.; Moll,J.L.; Francoual,M.; Berto,M.; Namer,M. Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours, *Br.J.Cancer*, **1987**, *55*, 509–512.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 500 ng IS in MeOH, mix, add 500 μL 3 M NaCl, add 1 mL water, mix, add to SPE cartridge, wash with 3 mL water, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:MeOH:water:1% ammonium chloride:1% potassium carbonate 950: 30:20:4:8
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: F ex 247 em 378 following post-column photochemical derivatization in a 15 m × 0.3 mm PTFE tube knitted on a mercury lamp (UV 254)

CHROMATOGRAM
Retention time: 6.8 (E isomer), 7.2 (Z isomer)
Internal standard: 1-(4-diethylaminoethoxy)phenyl-1,2-diphenylethanol (4.7)
Limit of detection: 0.4 ng/mL
Limit of quantitation: 1.25 ng/mL (E), 0.75 ng/mL (Z)

KEY WORDS
plasma; SPE; post-column photochemical derivatization

REFERENCE
Ürmös,I.; Benkö,S.M.; Klebovich,I. Simple and rapid determination of clomiphene *cis* and *trans* isomers in human plasma by high-performance liquid chromatography using on-line post-column photochemical derivatization and fluorescence detection, *J.Chromatogr.*, **1993**, *617*, 168–172.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Photolyze clomiphene in chloroform, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 33 (sic) 10 μm μPorasil
Mobile phase: MeCN:dichloromethane 20:80 containing 0.1% methenamine
Flow rate: 1.6

Injection volume: 20
Detector: UV 298

CHROMATOGRAM

Retention time: 9 (E isomer), 11 (Z isomer)

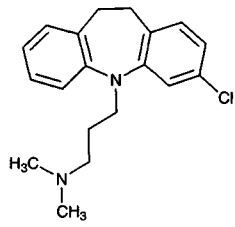
KEY WORDS

normal phase

REFERENCE

Frith,R.G.; Phillipou,G. Application of clomiphene photolysis to assays based on analysis of the derived phenanthrenes, *J.Chromatogr.*, **1986**, *367*, 260–266.

Clomipramine



Molecular formula: $C_{19}H_{23}ClN_2$
Molecular weight: 314.86
CAS Registry No.: 303-49-1, 17231-77-6 (HCl)
Merck Index: 2447

SAMPLE

Matrix: blood
Sample preparation: Add 500 μL 40 ng/mL IS in water to 500 μL plasma. Add to an activated 100 mg Bond-Elut C8 SPE cartridge. Wash with 1 mL water, 1.5 mL MeCN: water 50:50, and 500 μL MeCN. Elute with 1 mL MeOH, dry the eluate under a stream of nitrogen. Reconstitute the residue in 50 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Injection volume: 20
Detector: E, Coulochem 5100A, 5011 analytical cell +400 mV screen electrode, 700 mV sample electrode

CHROMATOGRAM

Internal standard: imipramine
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics; SPE

REFERENCE

Müller,F.O.; Schall,R.; Mogilnicka,E.M.; Groenewoud,G.; Hundt,H.K.L.; Luus,H.G.; Middle,M.V.; Swart,K.J.; De Vaal,A.C. Relative bioavailability of four clomipramine hydrochloride tablet products, *Biopharm.Drug Dispos.*, **1996**, *17*, 81–90.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM

Retention time: 18.87
Internal standard: clovoxamine (6.504)
Limit of quantitation: 7 ng/mL(UV 226, UV 400); 10 ng/mL(UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, desipramine, fluoxetine, imipramine, maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 5 M NaOH, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it under a gentle stream of nitrogen at 20 °, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RP C18 (Brownlee)
Column: 150 × 4.6 5 μm Microsorb MV
Mobile phase: MeCN:water 55:45 containing 10 mM pH triethylamine, adjusted to pH 4.8 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Retention time: 7.4
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: fluoxetine, norfluoxetine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Holladay,J.W.; Dewey,M.J; Yoo,S.D. Quantification of fluoxetine and norfluoxetine serum levels by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *704*, 259–263.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase (J.Liq.Chromatogr. 1981, 4, 849).

HPLC VARIABLES
Guard column: 23 × 3.9 Bondapak/Corasil C 18

Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 8.5
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: maprotiline
Simultaneous: amoxapine, amitriptyline, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine
Interfering: nortriptyline

KEY WORDS
clomipramine is IS; plasma

REFERENCE
Wong,S.H.Y.; Waugh,S.W. Determination of the antidepressants maprotiline and amoxapine, and their metabolites, in plasma by liquid chromatography, *Clin.Chem.*, **1983**, *29*, 314–318.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 4
Internal standard: desipramine hydrochloride (12)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: desmethylclomipramine, maprotiline, protriptyline, metabolites
Also analyzed: doxepin, desmethyldoxepin, amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

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KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Take 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: clomipramine

OTHER SUBSTANCES
Simultaneous: imipramine, desipramine, trimipramine
Noninterfering: amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, citalopram, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: diazepam

KEY WORDS
plasma; clomipramine is IS

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 8

Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine, imipramine
Also analyzed: diltiazem, nortriptyline, amitriptyline, haloperidol, propafenone, amiodarone, verapamil

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 27
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, doxepin, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 3 μL 20 ng/mL clobazam + 1 mL saturated sodium borate (adjusted to pH 11 with 6 M NaOH) + 5 mL n-hexane, mix 2 min, centrifuge at 3000 g for 10 min. Remove organic phase and evaporate to dryness under a stream of helium at 30°. Reconstitute in 20 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long Pelliguard LC-8 40 μm (Supelco)
Column: 150 × 4.6 C8 5 μm (Supelco)

Mobile phase: MeCN:buffer 50:50 (Buffer was 1.2 mL butylamine in 1 L 10 mM NaH_2PO_4, pH adjusted to 3 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.085
Internal standard: clobazam (k' 1.344)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: desipramine, nortriptyline, imipramine, amitriptyline
Simultaneous: nitrazepam, lorazepam, clonazepam, triazolam, flunitrazepam, alprazolam, diazepam, haloperidol, maprotiline

KEY WORDS
serum

REFERENCE
Segatti,M.P.; Nisi,G.; Grossi,F.; Mangiarotti,M.; Lucarelli,C. Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis, *J.Chromatogr.*, **1991**, *536*, 319–325.

SAMPLE
Matrix: blood
Sample preparation: For each 1 mL plasma or serum add 10 µL 14 µg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 20 × 4.6 10 µm Hypersil MOS C8; B 20 × 4.6 5 µm Hypersil CPS CN + 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 8.54
Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, desipramine, fluvoxamine, imipramine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol
Interfering: amitriptyline, doxepin

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL protriptyline in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 12.6
Internal standard: protriptyline (6.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)

Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 28.5
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES
Extracted: chlorpromazine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, imipramine, pipamperone, risperidone, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 500 ng/mL imipramine in EtOH + 500 μL 500 mM NaOH, mix, add to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 5 mL n-hexane:isoamyl alcohol 98:2. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Partisphere silica (Whatman)
Mobile phase: Hexane:EtOH:dichloromethane:diethylamine 77:18:5:0.003
Flow rate: 1.3
Detector: UV 214

CHROMATOGRAM
Retention time: 10
Internal standard: imipramine (3)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, desipramine, doxepin, fluoxetine

KEY WORDS
plasma; SPE; normal phase

REFERENCE
Altieri,I.; Pichini,S.; Pacifici,R.; Zuccaro,P. Improved clean-up procedure for the high-performance liquid chromatographic assay of clomipramine and its demethylated metabolite in human plasma, *J.Chromatogr.B*, **1995**, *669*, 416–417.

SAMPLE
Matrix: blood
Sample preparation: Stabilize plasma with 2 mg/mL sodium borohydride to prevent conversion of lofepramine to desipramine. 2 mL Plasma + 200 μL 1 M pH 11 sodium car-

bonate + 5 mL hexane:isoamyl alcohol 99:1, shake for 5 min, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long 5 μm Supelcosil LC-PCN
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:buffer 120:35:100 (Buffer was 2.07 g/L $NaH_2PO_4.H_2O$ in water.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 254

CHROMATOGRAM

Retention time: 3.46
Internal standard: clomipramine

OTHER SUBSTANCES

Extracted: lofepramine, desipramine
Simultaneous: nortriptyline, zuclopenthixol, imipramine, perphenazine, flupentixol, amitriptyline, haloperidol

KEY WORDS

plasma; clomipramine is IS

REFERENCE

Elm,T.; Hansen,E.L. Simultaneous determination of lofepramine and desipramine by a high-performance liquid chromatographic method used for therapeutic drug monitoring, *J.Chromatogr.B*, **1995**, *665*, 355–361.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 252

CHROMATOGRAM

Retention time: 12.00
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 25 μL MeOH, vortex for 30 s, add 100 μL 5 M NaOH, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb MV octadecyl

Mobile phase: MeCN:10 mM triethylamine 60:40, pH adjusted to 3.0 with 85% phosphoric acid

Flow rate: 1

Injection volume: 20

Detector: UV 260

CHROMATOGRAM
Retention time: 8.3
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: desipramine, imipramine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Dewey,M.J. Rapid microsample analysis of imipramine and desipramine by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1995**, *668*, 338–342.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 μL buffer + 50 μL 8 μg/mL IS in MeOH + 5 mL heptane:ethyl acetate 80:20, mix mechanically for 10 min, centrifuge at 3500 g for 10 min. Remove the organic layer and add it to 1 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3500 g for 10 min. Remove the aqueous layer and add it to 400 μL buffer and 5 mL heptane:ethyl acetate 80:20, extract, centrifuge at 3500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 150 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by adjusting pH of 8% sodium bicarbonate solution to 10.5 with 100 mM NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: MeOH:water 70:30 containing 7 mM butylamine
Flow rate: 1.2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: metabolite I of trimipramine (14)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chlorpromazine, flunitrazepam, levomepromazine, lorazepam, nitrazepam, oxazepam

KEY WORDS
serum

REFERENCE
Coudore,F.; Hourcade,F.; Molinier-Manoukian,C.; Eschalier,A.; Lavarenne,J. Application of HPLC with silica-phase and reversed-phase eluents for the determination of clomipramine and demethylated and 8-hydroxylated metabolites, *J.Anal.Toxicol.*, **1996**, *20*, 101–105.

SAMPLE
Matrix: blood, erythrocytes
Sample preparation: Evaporate 100 μL 10 μg/mL levallorphan in MeOH into a tube under a stream of nitrogen at 30°, add 1 mL plasma or erythrocytes, add 5 mL hexane:diethyl ether 80:20, shake mechanically for 10 min, centrifuge briefly at 4° at 3000 g, repeat

extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyanopropylsilane (Societe SGE)
Mobile phase: MeCN:MeOH:10 mM pH 7.0 potassium phosphate 60:15:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 25
Detector: UV 220

CHROMATOGRAM
Retention time: 7.3
Internal standard: levallorphan (6.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, amitriptyline, carbamazepine, clonazepam, diazepam, digoxin, fluvoxamine, haloperidol, imipramine, oxazepam, phenytoin, prazepam, salicylic acid, theophylline, thioproperazine, thioridazine, triazolam, valproic acid

KEY WORDS
plasma

REFERENCE
Marescaux,P.; Belan,E.; Houdret,N.; Goudemand,M.; Lhermitte,M. Simultaneous determination of clomipramine and its demethylated metabolite in plasma and erythrocytes by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2171–2177.

SAMPLE
Matrix: blood, tissue
Sample preparation: 100 μL Serum or 200 μL brain homogenate + 100 μL 5.0 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 5 min. Evaporate organic layer under a gentle stream of nitrogen at 20°. Reconstitute residue with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Microsorb MV C18 (Rainin, Woburn, USA)
Mobile phase: MeCN:water 55:45 containing 10 mM triethylamine, pH adjusted to 4.8 with 85% phosphoric acid
Flow rate: 1.0
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Internal standard: clomipramine

KEY WORDS
serum; brain; mouse; clomipramine is IS

REFERENCE
Holladay,J.W.; Dewey,M.J.; Yoo,S.D. Pharmacokinetics and antidepressant activity of fluoxetine in transgenic mice with elevated serum α-1-acid glycoprotein levels, *Drug Metab.Dispos.*, **1998**, *26*, 20–24.

SAMPLE
Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 32.1
Internal standard: cianopramine (8.93)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue

Sample preparation: Whole blood. 1 mL Whole blood + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, agitate gently for 30 min, centrifuge at 3500 rpm for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min, inject a 30 μL aliquot of the aqueous layer. Liver. Homogenize 10 g freshly minced liver with 10 mL water, adjust pH to 10 with 1 M NaOH, add 10 mg subtilisin (Sigma), heat at 55° for 1 h, adjust pH to 7.0 ± 0.5 with dilute mineral acid. 500 μL Homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, agitate gently, centrifuge for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate, centrifuge, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeCN:buffer 40:60 (Buffer was 100 mM potassium phosphate containing 2.5% diethylamine, pH 8.0.)
Flow rate: 2
Injection volume: 30
Detector: UV 220, UV 254

CHROMATOGRAM

Retention time: 32
Internal standard: cianopramine (8.9 ?)
Limit of detection: <200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Cordner,S.M.; Drummer,O.H. Postmortem clomipramine: therapeutic or toxic concentrations?, *J.Forensic Sci.*, **1994**, *39*, 486–493.

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 20.6 (A), 40.2 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: 500 μL Urine + 470 μL 3% L-(+)-ascorbic acid in 200 mM KH_2PO_4 + 30 μL β-glucuronidase/arylsulfatase (Boehringer Mannheim), vortex for 2 s, heat at 37° for 16 h to deconjugate, add 50 μL 2 M NaOH. 1 mL Plasma, urine, or deconjugated urine + 1 mL 600 mM pH 11.3 potassium carbonate + 100 μL 5 (plasma) or 20 (urine) μM desipramine in EtOH + 5 mL heptane:MTBE:n-butanol 47.5:47.5:5, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze at -50°. Remove the organic layer and add it to 1 mL 20 mM HCl, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Discard the organic layer and add 500 μL 600 mM pH 11.3 potassium carbonate to the thawed aqueous layer. Add 3 mL heptane:MTBE:n-butanol 47.5:47.5:5 to the aqueous layer, vortex for 1 min, centrifuge at 1400 g for 10 min, freeze. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeOH, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4 7 μm 120 Å Nucleosil

Column: 250 × 4 5 μm 100 Å Nucleosil RP-phenyl

Mobile phase: MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate + 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 25.33

Internal standard: desipramine (14.19)

Limit of detection: 10 nM (urine), 5 nM (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Nielsen,K.K.; Brosen,K. High-performance liquid chromatography of clomipramine and metabolites in human plasma and urine, *Ther.Drug Monit.*, **1993**, *15*, 122–128.

SAMPLE

Matrix: blood, urine

Sample preparation: 100 μL Serum or urine + 25 μL MeOH + 100 μL 5 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 5000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 10 × 4.6 5 μm Microsorb MV C18

Mobile phase: MeCN:10 mM triethylamine in water 60:40, pH adjusted to 3.0 with 85% phosphoric acid

Flow rate: 1

Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: clomipramine

OTHER SUBSTANCES
Extracted: desipramine, imipramine

KEY WORDS
mouse; serum; clomipramine is IS

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Baumann,H.; Dewey,M.J. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated α-1-acid glycoprotein, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 918–922.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.442

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract

with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluororacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Simultaneous: cimetidine, halofantrine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6

Injection volume: 20
Detector: UV 265

OTHER SUBSTANCES

Also analyzed: amitriptyline, chlorpromazine, promazine, promethazine, thymol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, prom-

azine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 17.90 (A), 8.53 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin,

trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

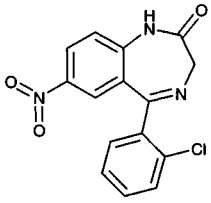
CHROMATOGRAM
Retention time: k' 3.99

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Clonazepam



Molecular formula: $C_{15}H_{10}ClN_3O_3$
Molecular weight: 315.72
CAS Registry No.: 1622-61-3
Merck Index: 2449

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.3 (A), 18.2 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 μL 512 nM IS in MeOH:water 5:95, add to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH. Elute with 200 μL and 100 μL MeOH, evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:MeOH:10 mM pH 3.7 K_2HPO_4 30:2:10
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 7.4
Internal standard: flunitrazepam (9.8)
Limit of detection: 5 nM

OTHER SUBSTANCES
Extracted: alprazolam, nitrazepam
Simultaneous: amobarbital, carbamazepine, citalopram, clobazam, clozapine, diazepam, doxepin, ethosuximide, norclobazam, oxazepam, oxcarbamazepine, pentobarbital, phenobarbital, phenytoin, primidone, valproic acid, zopiclone
Interfering: medazepam, midazolam, nordiazepam, temazepam

KEY WORDS
SPE; plasma; serum

REFERENCE
Åkerman,K.K.; Jolkkonen,J.; Parviainen,M.; Penttilä,I. Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction, *Clin.Chem.*, **1996**, *42*, 1412–1416.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.417

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.